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(54) PREPARATION OF POLYAMINE

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain an high-purity polyamine capable of providing food to which a polyamine is added, free from taste and odor, especially a nutrient composition for infants in large amounts without impairing the quality of food by treating yeast cell, cultured solution, etc., of yeast under acidic conditions.

SOLUTION: An acid solution such as 1N hydrochloric acid is added to a cell of a yeast such as Saccharomyces cerevisiae, Saccharomyces arbergensis or Candida utilis and/or cultured solution of the yeast so that pH becomes ≤2 and the yeast cell is physically subjected to crushing treatment and then subjected to acid extraction treatment and centrifuged to recover a supernatant and the supernatant is neutralized with 10-30% sodium hydroxide solution and then, the neutralized solution is passed through a column for gel permeation and a polyamine is fractionated from impurities by fractionation of molecular weight to provide the objective polyamine useful for foods, especially a nutrient composition for infants in high purity and large amounts without impairing quality of foods when it is added to foods.

LEGAL STATUS

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[The technical field to which invention belongs] this invention uses yeast or yeast culture medium as a raw material, and relates to the method of preparing polyamine in large quantities efficiently. It is related with the manufacture method of polyamine characterized by processing a yeast-fungus object and/or yeast culture medium under acid conditions in detail. Furthermore, it is related with the manufacture method of the polyamine addition food characterized by adding the polyamine of the yeast origin obtained by these methods. The polyamine obtained by this invention does not have a nasty smell taste, has composition still near polyamine composition of mother's milk, and can use it effective in food, especially the nutrition constituent for infants.

[0002]

[Description of the Prior Art] Polyamine is aliphatic hydrocarbon of the shape of a straight chain with two or more first-class amino groups, such as a putrescine, a spermidine, and a spermine. As the physiological function of polyamine. **, The stabilization and the structural change of a nucleic acid by the interaction with (1) nucleic acid, (2) The promotion operation to various nucleic-acid-biosynthesis systems, Activation of the synthetic system of (3) protein, acetylation of (4) histones, Phosphorization promotion of non-histone chromatin protein, stabilization of (5) cell membranes, and strengthening of the membrane permeability of the matter, It reaches. It is known that activation of the various enzymes which receive influence in (6) divalent metallic ions etc. is various (1266 now Kazutomo Hori, Tamio Yamakawa editorial supervision, a biochemistry dictionary, the 2nd edition, p. 1990). Moreover, polyamine which the effect which promotes multiplication and specialization of a cell is reported by recently, and carried out especially the ingestion, Promoting maturation of alimentary canal membrane is reported (Buts J.-P.et al., Digestive Diseases and Science, Vol.38, p1091 (1993); Grant, A.L.et al., J.Anim.Sci., Vol.68, p363 (1990); Dufour, C.et al., Gastroenterology, Vol.95, and p112 (1988)). Furthermore, the polyamine which carried out the ingestion is incorporated inside of the body promptly, and being used in an organization is also reported (Bardocz, S.et al., J.Nutr.Biochem, Vol.4, and p66 (1993)).

[0003] As an example which used polyamine for food, it is adding a spermine and a spermidine at the time of konnyaku manufacture. By blending the manufacture method (JP,6-38690,A) of konnyaku of not having a bad influence on other food even if there are few smells peculiar to konnyaku and it cooks with other food, and polyamine Moreover, it considers as the example which used polyamine as medical supplies. the polyamine

combination nutrition constituent (JP,6-305956,A) which is made to promote proteinic absorption and maintains good growth and good health condition -- The method of preventing gastric-acid secretion, the constituent for ingestion for gastric-acid secretion prevention (JP,58-131914,A), and an immunostimulator (JP,59-98015,A and JP,2-223514,A) are mentioned.

[0004] Thus, it is thought that it is desirable also from a nutritional viewpoint to strengthen to a nutrition constituent with few polyamine contents originally since various effects are known by polyamine and the importance is beginning to be recognized in recent years. However, the manufacture method of the high-concentration polyamine which can be used for food is not yet established. Many [for fermentation food, such as that it is few to milk or vegetables and bean paste, / meat and a cheese head have many contents in the food of polyamine, and] is known (Bardocz, S.et al., J.Nutr.Biochem, Vol.4, p66 (1993); polyamine study group, the collection of the 12th research presentation meeting lecture summaries, 4 pages, 1995). However, in order for nutrition constituents, such as modified milk powder for sucklings, to use cow's milk as the main raw material, since most polyamine is not contained, it needs to prepare the polyamine of the high grade which can be used for the modified milk powder for sucklings with few polyamine contents etc. in large quantities.

[0005] Proteolysis milk is mentioned as a nutrition constituent with high only and polyamine content. Buts and others reported that many polyamine was contained in the milk powder for puericulture which decomposed the source of protein with the rough refining enzyme of the pancreas origin (Buts, J.P.et al., J.Pediatr.Gastroenterol.Nutr., Vol.21, and p44 (1995)). In this case, polyamine was the crude enzyme origin used for the purpose of proteolysis, and was not a thing aiming at strengthening polyamine. Furthermore, you have to add, after decomposing those components in a constituent superfluously and refining polyamine as much as possible, since the enzyme which decomposes not only protein but a fat, sugar, a nucleic acid, etc. is contained in it when the crude enzyme of the pancreas origin is used for the usual constituent.

[0006]

[Problem(s) to be Solved by the Invention] As a result of this invention persons' searching wholeheartedly in quest of the high natural product of a polyamine content in view of these situations, it found out that a spermidine and a spermine are contained in yeast at high concentration, and having composition with the polyamine of the yeast origin very near the polyamine composition in mother's milk further. Furthermore, as a result of repeating research wholeheartedly paying attention to this polyamine, the method of it being efficient and preparing the polyamine of a high grade in large quantities was found out. Namely, this invention makes it a technical problem to offer the method of preparing polyamine in large quantities and efficiently by using a yeast-fungus object or yeast culture medium as a raw material. Furthermore, let it be a technical problem to offer the manufacture method of polyamine addition food without the nasty smell taste characterized by adding the polyamine of the yeast origin.

[0007]

[Means for Solving the Problem] this invention uses yeast or yeast culture medium as a raw material, and relates to the method of preparing polyamine in large quantities efficiently. It is related with the manufacture method of polyamine characterized by processing a yeast-fungus object and/or yeast culture medium under acid conditions in

detail. Furthermore, it is related with the manufacture method of the polyamine addition food characterized by adding the polyamine of the yeast origin obtained by these methods. The polyamine obtained by this invention does not have a nasty smell taste, and it can be used effectively, without spoiling the quality of food, even if it adds for food.
[0008]
[Embodiments of the Invention] as the yeast used as a raw material of this invention -- Saccharomyces A group and Candida the yeast belonging to a group and a Zyrosasccharomyces group mentions -- having -- especially -- desirable -- S.cerevisiae, S.carbergensis, S.uvarum, and S.diastaticus S.rosei C.utilis and Z.rouxii etc. -- it is mentioned These yeast-fungus object and its culture medium, and a further show the polyamine content in the natural product of mother's milk or others in Table 1.
[0009]
[Table 1]

	プトレッシン	スペルミジン	スペルミン	その他のポリアミン
人乳 (μg/100ml)				
泌乳期 3-5 日	2	40	80	n.d.
泌乳期31-60 日	3	50	80	n.d.
S. cerevisiae				
菌体 (μg/100g) *	511	4920	7015	507
培養液 (μg/100ml)	5	131	158	19
S. diastaticus				
菌体 (μg/100g) *	967	8320	11788	2113
培養液 (μg/100ml)	15	122	98	20
C. utilis				
菌体 (μg/100g) *	2898	50130	49051	1100
培養液 (μg/100ml)	5	193	250	28
サケ白子 (μg/100g) *	54060	n.d.	31291	199

(*：乾燥物100g当たり、n.d.：検出限界以下)

[0010] Polyamine composition of such yeast has the feature which is 70% or more in the sum total of a spermine and a spermidine, and is comparatively close to composition of mother's milk. Therefore, when it uses for the infant formula which is a mother's milk substitute, from a viewpoint of bringing close to mother's milk, it is very desirable. Although much polyamine is contained also in the albino of a salmon, since a spermidine is hardly contained, for the purpose of bringing close to polyamine composition of mother's milk, it is not desirable. Moreover, the polyamine content in the nutrition constituent currently generally sold is shown in Table 2. It is clear to the nutrition constituent for commercial infants that polyamine is hardly contained.
[0011]
[Table 2]

	プトレッシン	スペルミジン	スペルミン	その他のポリアミン (μg/100ml)
乳児用調製粉乳	n.d.	n.d.	n.d.	30
未熟児用調製粉乳	n.d.	n.d.	n.d.	25
フォローアップミルク	n.d.	n.d.	n.d.	20

(n.d.：検出限界以下)

[0012] Extraction of the polyamine from yeast can be performed as follows. That is, it is obtained by processing a yeast-fungus object and/or yeast culture medium under acid conditions. After refining the extract which added the acid solution and was obtained in detail after crushing the yeast-fungus object and homogenizing or adding an acid solution on a yeast-fungus object, it is obtained by refining the extract which performed spallation and homogenization, refined the obtained extract, or added the direct acid solution to yeast culture medium, and was obtained. At this time, centrifugal separation recovers the yeast-fungus object after liquid culture, and a biomass is destroyed by the physical crushing methods, such as a supersonic method and the French-press method, etc. Moreover, an autolysis method (use of organic solvents, such as toluene and ethyl acetate) which is used in case a yeast extract is manufactured can also be used. Although especially the culture medium used at this time in order to cultivate yeast is not limited, YM culture medium, a molasses culture medium, a malt extract culture medium, a potato, a glucose culture medium, etc. are especially mentioned, and a molasses culture medium and YM culture medium are desirable. Moreover, the yeast removed after beer fermentation can be used. Cultivation of yeast is cultivated about one to three weeks by 20-37 degrees C and pH 3-7. Then, an acid solution is added so that pH may become two or less, it stirs for about 0.5 to 6 hours, and 30-80 degrees C of polyamine are extracted. At this time, inorganic acids, such as 0.01-6-N sulfuric acid, a hydrochloric acid, an acetic acid, a phosphoric acid, trichloroacetic acid, perchloric acid, and a sulfosalicylic acid, are mentioned as an acidic solution. Subsequently, by centrifugal separation, it separates into a supernatant-liquid fraction and a sedimentation fraction, and each is collected. Although a fraction required for subsequent refining processings is a supernatant-liquid fraction, it adds an acid solution again also about a sedimentation fraction, and obtains a supernatant-liquid fraction by the same extract operation. Although polyamine is extracted by high concentration by these supernatant-liquid fractions, since protein other than polyamine, a lipid component, etc. may mix, to them, it is necessary to refine and condense further. this time -- a supernatant-liquid fraction -- a part for an ion-exchange method, a gel-filtration method, and a film -- the purity of polyamine can be raised by carrying out refining processing using any one or more methods of a drawing technique, an electrodialysis process, a solvent extraction method, or the heat-treating method

[0013] At this time, as an ion-exchange method, as long as an ion exchange group has a sulfonic group, a sulfo propyl group, a phosphoric-acid machine, a carboxyl methyl group, an aminoethyl machine, a diethylamino machine, the 4th class aminoethyl machine, the 4th class ammonium, etc., any are sufficient, by neutralizing and dipping the supernatant-liquid fraction obtained at each process, it adsorbs, and polyamine elutes and collects by acidic solutions, such as a sulfuric acid and a hydrochloric acid, further. since

[moreover,] molecular weight is large and the polyamine contained in a supernatant-liquid fraction differs from other components -- a part for a gel-filtration method or a film -- fractionation can be carried out by the drawing technique For a putrescine, 88 and a spermidine are [145 and the spermine of each molecular weight] 202. It is possible to carry out fractionation by gel filtration etc. on the other hand, since the protein of a macromolecule etc. is comparatively contained in the impurity in the supernatant-liquid fraction containing polyamine. The supernatant-liquid fraction obtained at each process is neutralized, and a gel-filtration method is dipped in the column filled up with gel-filtration support, it raises purity by molecular weight fractionation, and collects them. In addition, what is necessary is for which support, such as a dextran system, an acrylamide system, an agarose system, a cellulose system, a polyvinyl system, a textile glass yarn, and a polystyrene system, to be sufficient as gel-filtration support, and for the ranges of a cut off molecular weight just to be 100-100,000. Film fractionation methods are ultrafiltration membranes, such as a cellulose system, a cellulose acetate system, a polysulfone system, a polyamide system, a polyacrylonitrile system, a polytetrafluoroethylene system, a polyester system, and a polypropylene system, as membranogen material, and any film can be used for them if the ranges of a cut off molecular weight are 100-100,000. Moreover, if it aims at desalting, the usual permeable membrane can be used. An electrodialysis process supplies by turns the supernatant-liquid fraction and brine which were collected by the above-mentioned method between each film divided by the cation exchange membrane and the anion exchange membrane, and performs an electrodialysis. Initial-current density 0.5 - 15 A/dm2, voltage 0.1 - a 1.5V/tub are suitable for the condition. A solvent extraction method is a method of extracting polyamine using organic solvents, such as chloroform, amyl alcohol, and n-butanol, after making alkaline the solution extracted with the acid. The heat-treating methods are heat denaturation and the method of making it deactivate about the enzyme of the yeast origin currently mixed in a supernatant-liquid fraction. a supernatant-liquid fraction or refining -- it can be in process, in the case of-like [solution], temperature can be raised at 80-130 degrees C, and an enzyme can be made to deactivate In addition, if it processes combining such refining down stream processing, the polyamine of a high grade can be obtained more.

[0014] Furthermore, neutralization, dialysis, electrodialysis, or vacuum concentration removes the acid in the supernatant-liquid fraction obtained at each process if needed. Moreover, the supernatant-liquid fraction obtained at each process can be made powdered by liquefied or freeze drying, spray drying, etc., and is suitably chosen according to a use gestalt. Thus, since the obtained polyamine does not have a nasty smell taste and has composition still near the polyamine of mother's milk, it can be used effective in food, especially the nutrition constituent for infants. As the food which added the polyamine of this invention, or a nutrition constituent, although protein, a fat, sugar, vitamins, and minerals are constituted as a principal component, especially an infant formula can be mentioned. As an infant formula, the modified milk powder for premature babies etc. can be mentioned to the modified milk powder for matured infants, follow-up milk, and an allergy milk pan.

[0015] As protein, the hydrolyzate which processed casein, a milk-serum-protein concentrate, a milk-serum-protein separation object, milk-protein fractionation objects (alpha-casein, beta-casein, a beta lactoglobulin, alpha-lactalbumin, lactoferrin, etc.),

soybean proteins, and also such protein by the protease or the peptidase can be used. As a fat, it can use besides vegetable fat and oil, such as microorganism oils, such as animal fat and oil, such as fish oil, lard, and milk fat, yeast, and algae, and soybean oil, combining suitably these judgment oils, a hydrofined oil, an ester-interchange oil, etc. Starch, fusibility polysaccharide, a dextrin, a sucrose, a lactose, grape sugar, an oligosaccharide, etc. can be used for sugar. "A vitamin and a mineral CODEX specification and the related sanitation work rule containing Japanese international dairy league issue and infant food of particular-application food, CAC/VOL.IX - The 1st edition and Supplement 1, 2, 3, 4" (1993), Food, and "science company issue, a 1993 edition specification item food additive handbook (the 31st edition of amendment)" (1993), One or more sorts of usable things are used for food, and "science company issue, and a report-to-authorities food additive and a food material natural-product handbook (the 12th edition)" (1992) among the vitamin of a publication, and a mineral at an infant formula. As for the rate of a compounding ratio of protein, a fat, sugar, a vitamin, and a mineral, it is desirable to consider as per [of 5 - 40 % of the weight of each] solid, 5 - 40 % of the weight, 30 - 80 % of the weight, 0.005 - 5 % of the weight, and 0.005 - 5 % of the weight.

[0016] Suitably, 0.1mg - 500mg per 100g of nutrition constituents of polyamine manufactured by this invention is blended so that it may become the polyamine content of 0.2-20mg. What is necessary is just to add the loadings about 0.001 to 10% of the weight to the solid of a nutrition constituent, although based also on the purity of polyamine. As stated also in advance, since a spermidine and a spermine become a total of 70% or more and the composition can also be approximated to mother's milk in the polyamine prepared from yeast, the polyamine of mother's milk level is securable by both the amount per solid, and composition to the solid of a nutrition constituent adding the polyamine of this invention about 0.001 to 10% of the weight.

[0017]

[Example] Although the following examples explain this invention to a detail more, it is only only illustrating these and this invention is not limited at all by these.

[0018]

[Example 1]

Manufacture and 1C.utilis of polyamine Shell polyamine was prepared. That is, after cultivating in YM culture medium, 3l. of water was added to fully rinsed C.utilis 1kg (wet weight), and physical spallation was performed. 3l. of 1-N hydrochloric acids was added to this, and 40 degrees C of acid extraction were performed for 2 hours, stirring. It checked that pH at this time was two or less. After the extraction end, centrifugal separation was carried out and supernatant liquids were collected. After a sodium-hydroxide solution neutralizes a supernatant liquid 10 to 30%, it is the support for gel filtration (Superose12 and Sephadex G-25F, and Pharmacia Biotech K.K.) about neutralization liquid further. Fractionation of polyamine and the impurity was carried out to the column with which it was filled up by through and molecular weight fractionation. It freeze-dried, after carrying out heat sterilization processing of this fractionation liquid for 121 degrees C and 2 seconds. As a result of measuring the amount of polyamine contained in the obtained polyamine manufacture object according to the method (a Japanese child-nutrition digestive organ disease society magazine, nine volumes, 115-121 pages, 1995) of upper parts of rivers, 400mg of polyamine was contained in 1g of

obtained polyamine manufacture objects. In addition, 370mg of the spermines and spermidines in a polyamine manufacture object were contained in total.
[0019]
[Example 2]
Manufacture and 2S.cerevisiae of polyamine Shell polyamine was prepared. That is, after adding 4l. of 2-N sulfuric acids to 2kg (S.cerevisiae) of dry yeast and performing physical spallation, 35 degrees C of acid extraction were performed for 5 hours, stirring. It checked that pH at this time was two or less. After the extraction end, centrifugal separation was carried out and supernatant-liquid fractions were collected. It is a cation exchange resin (Dowex 50-X 8 (H+ type), the Muromachi chemical-industry company) about a supernatant liquid. Through and polyamine were made to stick to the column with which it was filled up. 0. After 5M brine's having fully washed the resin and removing an impurity, polyamine was eluted with 6-N hydrochloric acid. After adding the sodium-hydroxide solution to the eluate 10 to 30% and neutralizing, it desalted with the electrodialyzer (the micro reed riser S1, Asahi Chemical Industry Co., Ltd., film cartridge:AC-121-10), and the polyamine concentration fraction was obtained, and this fractionation liquid was freeze-dried after carrying out 121 degrees C and heat sterilization processing for 3 seconds. As a result of measuring the amount of polyamine contained in the polyamine manufacture object obtained by the same method as an example 1, 200mg of polyamine was contained in 1g of obtained polyamine manufacture objects. In addition, 180mg of the spermines and spermidines in a polyamine manufacture object were contained in total.
[0020]
[Example 3]
Manufacture and 3S.carbergensis of polyamine Shell polyamine was prepared. That is, acid extraction was performed, having added 3l. of 1-N sulfuric acids, and stirring them 35 degrees C for 4 hours, after adding 3l. of water to S.carbergensis 1kg (wet weight) and performing physical spallation. It checked that pH at this time was two or less. After the extraction end, centrifugal separation was carried out and supernatant liquids were collected. It is a cation exchange resin (Dowex 50-X 8 (H+ type), the Muromachi chemical-industry company) about a supernatant liquid. Through and polyamine were made to stick to the column with which it was filled up. 0. After 8M brine's having fully washed the resin and removing an impurity, polyamine was eluted with 5-N sulfuric acid. After adding the sodium-hydroxide solution to the eluate 10 to 30% and neutralizing, it desalted by the permeable membrane and the polyamine concentration fraction was obtained. This fraction was freeze-dried. As a result of measuring the amount of polyamine contained in the polyamine manufacture object obtained by the same method as an example 1, 90mg of polyamine was contained in 1g of obtained polyamine manufacture objects. In addition, 70mg of the spermines and spermidines in a polyamine manufacture object were contained in total.
[0021]
[Example 4]
Manufacture and 4C.utilis of polyamine Shell polyamine was prepared. Namely, C.utilis It cultivated using the same culture medium as an example 1, 50l. of 2-N sulfuric acids was added to 10l. of the culture medium, and the homogenate was prepared with the homogenizer. Acid extraction was performed stirring 40 degrees C of this homogenate

for 4 hours. It checked that pH at this time was two or less. After the extraction end, centrifugal separation was carried out and supernatant-liquid fractions were collected. After adding the sodium-hydroxide solution to the supernatant liquid 10 to 30% and making it alkaline, the solvent extraction was performed in n-butanol. The extract was condensed by the rotating evaporator and the solvent was removed. After neutralizing concentration liquid, it desalted with the electrodialyzer (the micro reed riser S1, Asahi Chemical Industry Co., Ltd., film cartridge:AC-121-10), and the polyamine concentration fraction was obtained and it freeze-dried. As a result of measuring the amount of polyamine contained in the polyamine manufacture object obtained by the same method as an example 1, 35mg of polyamine was contained in 0.5g of obtained polyamine manufacture objects. In addition, 30mg of the spermines and spermidines in a polyamine manufacture object were contained in total.

[0022]

[Example 5]

20kg and 93kg addition dissolution of 470kg of manufacture skimmilks of polyamine addition milk powder and the milk-serum-protein concentrate (WPC; Denmark protein company) of lactoses are carried out. this -- a water-soluble-vitamin component (vitamin B1, B-2, B6, and B12 and C --) A niacin, a folic acid, pantothenic acid, a biotin, a choline, an inositol, etc. and mineral components (a calcium carbonate, potassium chloride, magnesium sulfate, ferrous-citrate sodium, a copper sulfate, zinc sulfate, etc.), respectively 1kg, The liquid which suspended and dissolved 4g of polyamine manufacture objects prepared in the example 1 at 300g of warm water, And fat soluble vitamins (vitamin A, D, E and K, beta carotene, etc.) are dissolved. Linolic acid, gamma-linolenic acid, an arachidonic acid, alpha-linolenic acid, EPA (eicosapentaenoic acid) and DHA (docosa-hexaenoic acid), and 47.8kg of manufacture fats containing cholesterol are mixed. It homogenized. The obtained solution was sterilized and it condensed by the conventional method, and it dried and 200kg of milk powder was obtained. In addition, this milk powder was dissolved in warm water, and about 100microg / 100ml of polyamine were contained in the modified milk adjusted to 13% of rates of a solid.

[0023]

[Example 6]

the solution which dissolved manufacture WPC90kg and 550kg of lactoses of polyamine addition milk powder in warm water, and dissolved casein 75kg in this with the alkali of the specified quantity -- a vitamin -- and -- and the liquid which suspended and dissolved 40g of polyamine manufacture objects which prepared the mineral component (an example 5 -- the same) in 10kg and the example 3, respectively at 1000g of water and 239kg of manufacture fats which dissolved the same fat soluble vitamins as an example 5 -- mixing . It homogenized. The obtained solution was sterilized and it condensed by the conventional method, and it dried and 1000kg of milk powder was obtained. In addition, this milk powder was dissolved in warm water, and 100microg / 100ml of polyamine were contained in the modified milk adjusted to 13% of rates of a solid.

[0024]

[Effect of the Invention] From the above result, yeast or yeast culture medium is used as a raw material by this invention, and the method of preparing polyamine in large quantities efficiently is offered. In detail, a high grade and the method of preparing in large quantities are provided with polyamine by processing a yeast-fungus object and/or

yeast culture medium under acid conditions. Furthermore, the manufacture method of the polyamine addition food characterized by adding the polyamine of the yeast origin obtained by these methods is offered. The polyamine obtained by this invention does not have a nasty smell taste, and it can be used effectively, without spoiling the quality of food, even if it adds for food.